



Fig. 1

Figure 2

Mutation	Exemplary Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')	E_{300} M ⁻¹ cm ⁻¹
2789+5G>A	1	none	invader	TTTGGTTGTGCTGGCTCCCTTGGAAAGTGAT	330800
2789+5G>A	1	hex	probe/DM	CGCGCGGAGATATTCATGTCCTATTTGG	306500
2789+5G>A	1	none	synthetic target	CAATACCAATAGGCAATGGAATATACCTTCCAAAGGAGCCACGACACAACCAA	667000
R1162X	1	none	invader	GTTTACCTCTCTGTGGCATGTCAATGAACCTAAAGACTCT	428000
R1162X	1	hex	probe/DM	CGCGCGGAGGAGCTCAGATGCGC	253000
R1162X	1	none	synthetic target	TCAGATGGAGCTGTGAGCTGATGCTTTAAAGTTCATTGACATGCCAACAGAAAGGTTAAAC	659000
R347P	4	none	invader	CAGGGAATTGGCGAGTGCACGCCATGT	306600
R347P	4	hex	probe/ER24	ACGGACGCGGAGGCGAGCAACAATGCAG	318200
R347P	4	none	synthetic target	CTCATCTGCATGTTCTGCCCATGTGGCGTCACTGGGCAATTTCCCTGGG	488000
1898+1G>A	1	none	invader	GACTCTCTTTTGGATACCTAGATGTTTAAACAGAAAAGAAATATTTGAAAGT	619000
1898+1G>A	1	hex	probe/DM	CGCGCGGAGGATATGTTCTTTGAATACCTTACTTAT	386500
1898+1G>A	1	none	synthetic target	ATAAGTAGGTATTCAAAGACATATCTTCAAGACATATTTCTTTCTTTAAACATCTAGGTATCCAAAAAGGAGATC	904800
2184delA	4	none	invader	CCCCAACTCTCCAGTCTGTTTAAAGATATTTTTTC	393000
2184delA	4	hex	probe/DM	CGCGCGGAGGTTTGTGCCAGGAGACA	305200
del507	1	none	invader	GCTTTGATGAGCTTCTGTATCTATATTCATCATAGSAAACACCAAT	509300
del507	1	hex	probe/DM	CGCGCGGAGGATATTTCTTTAATGTTGCC	345200
del507	1	none	synthetic target	GCCTGGCAGCTAAAGAAATATCTTTGGTGTTCCTATGATGAATAGATACAGAGCGTCATCAAAGCATGCC	866600
G85E	4	invader	invader	GGCTTTGGCGAGTGTTTTCTGGAGATTAATGTTCTATGT	409100
G85E	4	hex	probe/ER24	ACGGACGCGGAGAAATCTTTTATATATAGGGGTAAAG	431700
G85E	4	none	synthetic target	AGATCTTACCTCAAAATAATAAAGAGTTTCATAGAACATAAATCTCCAGAAAAACATCGCGAAGGGCAATTA	869300
R117H	3	none	invader	AATCATAGTCTCTACGCCGGATAACAAGAGGAACT	443800
R117H	3	hex	probe/DM	CGCGCGGAGACTCTACGGGATTAATCT	304200
R117H	3	none	synthetic target	ATGCCATGAATATCGCGATGAGTGTTCCTCTGTATCCGGGTCTATAGGAAGCTATGATT	681700
R560T	1	none	invader	CATGAATGACATTTACAGCAATGCTTCTAGACCAATAATATAGTTATTCAT	595000
R560T	1	hex	probe/ER24	ACGGACGCGGAGTGTCTAAAGAAATCTTGCT	378100
R560T	1	none	synthetic target	GCAATTTGGATGACCTTCTGCCCTTACCATAATTTGACTTCATCCAGT	945400
3120+1G>A	2	none	invader	CGCGCGGAGGATATGTAATAAAGATACCGTTAA	496000
3120+1G>A	2	hex	probe/DM	CGCGCGGAGGATATGTAATAAAGATACCGTTAA	397500
3120+1G>A	2	none	synthetic target	AGACATATCAACGGTACTATTTTACATATCTGATGAAGTCAAAATGTTAGAGGCGAAGGTCATCCAAAATTGCTATATC	984000
3659delC	2	none	invader	GAGAGTGGCATCTGTATGTGGTTGGTTCAGTT	372300
3659delC	2	hex	probe/DM	CGCGCGGAGGTAGTTACTCTCTGTGG	302800
3659delC	2	none	synthetic target	CATGCCAACAGAGGTAAACCTACAGTCAAGTCAAGCAACCAACCAACCAAGATGGCCAACTCTC	679800
A455E	1	none	invader	CTTGAAAGATTAATTTCAAAGTAAAGAGGACGAGTGTGTGGT	531000
A455E	1	hex	probe/ER24	ACGGACGCGGAGGTTGCTGGATGCA	298100
A455E	1	none	synthetic target	CCAGTGGATCCAGCAACCTGCCCTCTTCTATCTTGAAATTAATCTTTCAGG	681000
1078delT	2	none	invader	AGTGATAGGGAACAGACATAAAACACACAT	413500
1078delT	2	hex	probe/DM	CGCGCGGAGGAAACCCGTGAAGAAGAA	355400
1078delT	2	none	synthetic target	AGCCTCTCTCTCGGCTCTGTGGTGTTTATCTGTGCTTCCCTATGCACT	533300
G551D	2	none	invader	CGAGAGAAAGACAATAGTCTTGAGAAAGTGGAAATCACACTGAGTGGAGT	628200
G551D	2	hex	probe/DM	CGCGCGGAGATCAACAGCAAGAAATTTCT	343800
G551D	2	none	synthetic target	CTTGTAAAGAAATCTTGTGCTGTTTGTCCACTCAGTGTGATTCACCCTTCTCCAAAGCACTATATGTCTTCTTGCACAACTT	883100
I148T	1	none	invader	AAATCAAACTAAACATAGCTATCTCATCTGCATTCAT	432400
I148T	1	hex	probe/ER24	ACGGACGCGGAGGTGTGATGAAGGCCAA	350200
I148T	1	none	synthetic target	CCATTTTGGCTTCATCACACTGGAATGCAGATGAGAAATAGCTATGTTAGTTTGATT	643100
N1303K	2	none	invader	CCATATTTCTTGATCATCTCCACTGTCTATAGGGATCCAA	414700
N1303K	2	hex	probe/DM	CGCGCGGAGCTTTTCTTAAATGTTCCAGAAA	391200
N1303K	2	none	synthetic target	ATTATTTTCTTGGAACTATAGAAAAGTTGGATCCCTATGAACAGTGGAATGATCAAGAAATATGGAAG	867100
711+1G>T	2	none	invader	CGCTTCCAGTGTATTAATTAACAAATAGTGCCTAAAAGATAAATAGGTACATT	
711+1G>T	2	hex	probe/DM	CGCGCGGAGAAATCATCAAATTTGTTGAGT	
711+1G>T	2	none	synthetic target	ACCTGAAACAAATTTGATGAATATGATACCTATTTGATTTAATCTTTTAGGCACTTGTATTAATCTTTTAAATATACAACTGGAAAGGC	927000
1717+1G>A	3	none	invader	GGCTTCAAACTCAGATTTGAGCATCTAAAGTGAAGTCTCTAATTTGTAATTTTGGTAAT	685000

٤٤ ٤٥

1717-1G-A	3	hex	probe/DM	CGGCGCGAGGAGACATCTCCAAAGTTGC	CGGCGCGAGGAGACATCTCCAAAGTTGC	294500
1717-1G-A	3	none	synthetic target	CTCTCAAACTTGGAGATGTCCTATACCAAAATTAAGAAATTAAGACAGTCACTTTAGTATGCTCAATCTGAATTTGAAAGGCACATC	1010000	
W1282X	3	none	invader	GCTCATCTGTGATCACTCACTCAAGGCTTTCTCTTA	345000	
W1282X	3	hex	probe/DM	CGGCGGAGGTCACTTGTGCAAAGTTATG	327800	
W1282X	3	none	synthetic target	GATTCAAATACTTCCACAGCTGAGGAAAGCCCTTTGGAGTGATACACACAGGTGAGCAA	683000	
3849+10kbC-T	2	none	invader	CAAGAGTCTTCCATCTGTTGCGATATATAAATGGA	390000	
3849+10kbC-T	2	hex	probe/DM	CGGCGGAGGTGAGTAAGACCCCTGAAA	327400	
3849+10kbC-T	2	none	synthetic target	TTCTTTCAGGGTGCTTACTCAACCATTTAATACTGCAACAGATGGAAGACTCTTG	601000	
R553X	4	none	invader	CATTACACGAATGCTGCTAGACCAATAATAGTTATTCACCTTGTCTAAAGAAATTTCTTGCTG		
R553X	4	hex	probe/DM	CGGCGGAGGCACTGACCTCCACTCAGT		
R553X	4	none	synthetic target	ACTGATGTGAGTCAATGAGCAAGATTTCTTTAGCAAGGTGAATACTAATATTGGTCTAGCAAGCATTTTGTCTGTAATG		
G542X	4	none	invader	TCCAAAGTTTCGAGAGAAAGACAATATAGTCTTTTC		
G542X	4	hex	probe/DM	CGGCGCGAGGAGGAGGTGGAATCACA		
G542X	4	none	synthetic target	TGTGATTCACTCTTCTCAAAAGCATATATTGTTCTTCTGCAAACTTGA		
621+1G-T	3	none	invader	CTTTCATCACATTTGGAATGAGATGAGATGCTTATGTTAGTTTGAATTAAGAAGC	664000	
621+1G-T	3	hex	probe/DM	CGGCGGAGGTAACTACTCTTGTGCACGG	311900	
621+1G-T	3	none	synthetic target	GGGCGCTGTGCAAGGAATATTACTCTTATAAATCAAACTAAACATAGCTATCTCATCTGCATTCGAATGTGATGAAGGCCAA	965000	
R334W	2	none	invader	CGCAGCAAAATGCAAGAATGAGATGGTGTGAATATTTTCCT	467000	
R334W	2	hex	probe/DM	CGGCGGAGGAGGAGGATGCTCTTTGATTA	336800	
R334W	2	none	synthetic target	TGCACATAATCAAGGAATCATCTCTCGAAATAATTCACACCATCTCATCTGCAATTTCTGCTGCG	703000	

Mutation	Exemplary Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')	ε_{260} $M^{-1} \text{ cm}^{-1}$
Internal control	all	none	Invasder	IgtaGtcaTgcTctcTacaCaagagaGaagagagacacaca	503500
Internal control	all	hex	Probe/SNP4b	Tccggcggttcctgaaggagaccaccaattcag	321200
Internal control	all	none	Synthetic Target	tttcagatgggtgtcttcctcagtgctctctcattctcttagtgtaaacancactaaagtacattt	698200

	all	1, 2	5	all
DM/FAM	Hex	Hex	Hex	Hex
ER24/FAM	Hex	Hex	Hex	Hex
SNP45/Red	Hex	Hex	Hex	Hex

X = Quencher = Z28

X = Quadrant = 440
Y = Dye = FAM for 1055-48-08 and 1055-48-09 and Y = Z35 (or Redmond Red) for 1055-49-04

Mutation	Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')	E_{260}^{-1} M ⁻¹ cm ⁻¹
delf508	delf508	none	Invasder	TGATGACGCTTCTGTATCTATCTATCATCATGAGGAACACA	441500
delf508	delf508	Hex	WT Probe	CGCGCCGAGCAAGATGATATTTCTTTTAATGCT	382200
delf508	delf508	Hex	Mut Probe	AGCTGTCGCGACACAATAATTTCTTTAATGTTGCCA	418100
delf508	delf508	Hex	DM/FA	Y-ctd-X- <i>sgc-egg</i> -lft-tcc-ggc-tga-gac-cle-ggc-ggc-hex	347150
delf508	delf508	Hex	Wingtail/Red	Y-ctd-X- <i>leg-gcc-lft-leg-ggc-aga-gat-gat-ggc-cga-gat-hex</i>	390400
delf508	WT Target	none	WT Target	TGCTCGCACCATTAAGAAAATATCATCTTTTGTTGCTTCGTATGAAATAGATACAGAAGGTCATCAAA	837500
delf508	delf508	none	Mut Target	ATGCTCTGGCACCATTAAGAAAATATCATCTTTTGTTGCTTCGTATGAAATAGATACAGAAGGTCATCAAAA	828100

Mutation	Pool	3' blocking group	Oligo type (Arm)	Sequence (5'-3')
2184delA	2184delA	none	invader	CTTCCTTTTTTCCCAAACTCTCCAGTCTGTTTAAAAGATTGTTTA
2184delA	2184delA	hex	MUT probe/DM	CGGCCGAGGTTTGTTCTTCTGTCAGGAG
2184delA	2184delA	hex	WT probe/ER24	ACGGACGCGGAGTTTGTCTGTCGCCAGGAG

X = Quencher = Z28

Y = Dye = FAM for 1055-48-08 and Y = Z35 (or Redmond Red) for 1144-16-02

711+1G-T	2	hex	probe/ER24
3849+10kh	2	hex	probe/ER24

Fig. 3

A

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
2789+5G>A	26mix	3.94	4.69	1.19
R1162X	29	3.42	2.18	0.62
R347P	15	3.38	4.60	1.36
G85E	21	3.62	2.55	0.70
R560T	9	3.30	2.47	0.75
delI507	1	3.16	1.98	0.63
1898+1G>A	111 A2/8	6.23	2.84	0.46
R117H	30	3.46	1.87	0.54
delF508 homo MT	3	3.44	1.14	0.33
WT gDNA	03-243	3.58	1.06	0.30

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
2184delA plasmid/internal control syn. Target	plasmid/syn. Target	4.67	3.65	0.78

B

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
A455E	8	3.26	2.88	0.88
3659delC	14	3.38	2.36	0.68
N1303K	16	3.92	2.11	0.54
3120+1G>A	6	3.84	2.45	0.64
G551D	20	3.44	2.04	0.59
WT gDNA	03-243	3.74	1.00	0.27

I148T/Internal control	syn. target	4.35	5.08	1.17
1078delT/Internal control	syn. target	4.44	4.97	1.12

C

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
711+1G>T	2	3.95	2.82	0.71
W1282X	19	4.44	2.16	0.49
1717-1G>A	28	4.87	2.19	0.45
3849+10kbC>T	5	3.82	2.48	0.65
WT gDNA	03-243	4.67	1.10	0.24

D

Mutation	Sample	IC ALLELE	MUT ALLELE	FOZ Ratio
621+1G>T	11	4.23	2.05	0.49
G542X	18	3.40	2.83	0.81
R553X	7	4.53	3.27	0.72
R334W	22	3.72	2.79	0.75
WT gDNA	03-243	4.18	1.14	0.27